ESTONIAN UNIVERSITY OF LIFE SCIENCES
Institute of Veterinary Medicine and Animal Sciences

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PREVALENCE AND COUNTS OF *CAMPYLOBACTER* SPP. IN POULTRY MEAT AT ESTONIAN RETAIL LEVEL

CAMPYLOBACTER SPP. LEVIMUS JA ARVUKUS LINNULIHAS EESTI JAEMÜÜGI TASANDIL

Final Thesis in Veterinary Medicine

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LÜHIKOKKUVÕTE

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<tr><td colspan="4">Campylobacter jejuni ja Campylobacter coli on ühed kõige levinumad bakteriliigid, mis põhjustavad inimeste soolestiku infektsioone Euroopa riikides. Värske kanabroileri liha on kõige olulisem Campylobacter spp. allikas, mis põhjustab enteraalseid infektsioone inimestel. Eestis on kampülobakterite levimus värskes linnulihas enamiku ELi riikide ja teiste Balti riikidega võrreldes suhteliselt madal. Antud uuringus tuvastati Campylobacter spp. kõrge levimus Läti ja Leedu päritolu värskes kanabroilerilihas, eriti Leedu päritolu broilerilihas. Kõik proovid osteti suuremate Eesti jaemüügi kettide poodidest.
Uuringu tulemuste analüüsi järel võib järeldada, et Leedu päritolu värske kanabroileriliha kujutab Eesti tarbijatele suuremat kampülobakterioosi riski, võrreldes Eesti ja Läti kanabroileri tootmisest saadud värske kanabroilerilihaga. Uuringus selgus statistiliselt oluline erinevus kampülobakterite levimuses Eesti päritolu ning Läti ja Leedu päritolu toodetes. Eesti päritolu kanabroilerilihast kampülobaktereid ei tuvastatud.</td></tr>
<tr><td colspan="4">Märksõnad: Campylobacter spp., levimus, arvukus, värske kanabroileri liha, jaemüük</td></tr>
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SUMMARY

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Kreutzwaldi 1, Tartu 51014</td><td colspan="2">Abstract of veterinary medicine thesis</td></tr>
<tr><td colspan="2">Author: Viorel Revenco</td><td colspan="2">Curriculum: Veterinary medicine 2013</td></tr>
<tr><td colspan="4">Title: Prevalence and counts of Campylobacter spp. in poultry meat at Estonian retail level</td></tr>
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Place and date: Tartu, 2019</td></tr>
<tr><td colspan="4">Campylobacter jejuni and Campylobacter coli are the most common bacteria causing of human intestinal infections in European countries, and fresh broiler chicken meat is the most important source of Campylobacter spp. which causes enteric infections in human.
In Estonia, compared with most EU-country and other Baltic countries the prevalence of Campylobacter has been relatively low.
In present study high prevalence of Campylobacter spp. in fresh broiler chicken meat of Latvian and Lithuanian origin was found. All samples were purchased at Estonian retail level. Campylobacter was not found from fresh broiler chicken meat of Estonian origin. It can be deduced that Lithuanian origin fresh broiler chicken meat may pose higher risk for campylobacteriosis for Estonian consumers compare to fresh broiler chicken meat originating from Estonian and Latvian broiler chicken meat production.</td></tr>
<tr><td colspan="4">Keywords: Campylobacter spp., prevalence, numbers, fresh broiler chicken meat, retail</td></tr>
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INTRODUCTION

The name of *Campylobacter* is derived from Greek word " Kampylos " which means curved, and " baktron " means rod, *Campylobacter* was first time described by Theodor Escherich in 1886, and isolated by John McFadyean in 1906, and genus name was established further in 1963 by Sebald and Véron (CDCP 2013).

Campylobacter spp. are a leading cause of bacterial enteritis in Europe (Spina *et al*. 2015), and based on Disability-Adjusted Life Year (DALY) estimations is also one of the most expensive foodborne diseases in Europe (Mangen *et al.* 2015). In the European Union in 2009, 201,711 *Campylobacter* cases were reported, and this number increased to 214,779 in 2013 (EFSA 2016), and was 246,307 in 2016 in the EU (EFSA 2017). *Campylobacter* spp. has been recognized to cause of bacterial gastroenteritis world-wide (Nachamkin *et al.* 2008). The report "The global poultry trends" (2014) described that poultry meat production has increased from 58.5 million tons in 2000 to 95.5 million tons in 2014, and the main reservoir of *Campylobacter* is poultry. Human can contact the disease from eating food contaminated with *Campylobacter* species or contact the infected animal (The global poultry trends 2014).

The most important primary contamination site of *Campylobacter* is at farm level because campylobacters exist widely in the outside environment of rearing halls (Jacobs-Reitsma 2000). Studies have shown that the main source of *Campylobacter* contamination of poultry carcasses is their intestinal content (Mead *et al.* 1995). Several epidemiological studies have shown that handling or eating poultry meat is a factor of campylobacteriosis (Friedman *et al.* 2004). *Campylobacter jejuni and C. coli* are the most common bacteria causing of human intestinal infection (Hänninen *et al.* 2003). It is also known that cross-contamination from raw poultry meat to other foods can also be an important source of infection (Kapperud *et al.* 2003). According to Altekruse *et al.* (1999), it was mentioned that the risks related to human infection can be reduced by proper cooking and handling of food.

Present work aims to study the prevalence and numbers of *Campylobacter* spp. in poultry meat at retail level in Estonia. Estonian, Lithuanian and Latvian products were sampled and analyzed, because these are the only fresh chicken meat products sold at Estonian retail level. The sampling in the present study was organized in manner which able to obtain comparable data of *Campylobacter* contamination of fresh broiler chicken meat at Estonian retail level.

ACKNOWLEGMENTS

I thank my supervisor's Professor Mati Roasto and Associate Professor Kadrin Meremäe, and all related staff from the Chair of Food Hygiene and Veterinary Public Health of Estonian University of Life Sciences who shared with me their professional knowledge how to conduct *Campylobacter* spp. prevalence and enumeration studies for broiler chicken meat.

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1. REVIEW OF THE LITERATURE

1.1 General description of *Campylobacter* spp.

Campylobacteriosis is an infection disease of several mammals including humans, ruminants, horses, and other poultry, caused by Family Campylobacteraceae mostly *Campylobacter jejuni* and *C. coli* species. *Campylobacter* spp. is a gram negative bacteria which typically appear spiral, or rod-shaped and motile. These bacteria are microaerophilic (growth optimum 85% N_2, 10% CO_2 and 5% O_2), which mean that these bacteria can grow both aerobically and anaerobically, but favor microaerobic conditions (Roasto 2008).

Nagulukun (2017) described that between 2000 and 2009, several new species of *Campylobacter* spp. ware reported like: (*C. lanienae*, *C. hominis*, *C. avium*), and from 2010 to 2015 six more new species was added to genus *Campylobacter*, which result at present time genus *Campylobacter* to contain 27 species and 8 subspecies. Kaakoush *et al*. (2015) mentioned that the genus *Campylobacter* contains 30 taxa, which 17 species are related to public health. *Campylobacter* grow optimally in an atmosphere with 5% O_2, although a concentration of 10% O_2 is sufficient to grow *Campylobacter* strains (Bolton and Coates 1983), an optimal temperature for growth between 30 to 45 ºC.

Hilbert *et al*. (2010) demonstrated that *C. jejuni* can survive aerobically for at least 48 h, when cultivated together with *Pseudomonas putida*. Also, these authors suggested that *Campylobacter jejuni* become more resistant to different stress situation, including higher O_2 concentration while growing together with *Pseudomonas putida*. Garénaux *et al.* (2008) mentioned that the *Campylobacter* can also be found in poultry meat, at temperature of +4 to +5 ºC, which are normal temperature for chilled storage of food products. According to Roasto (2008), *Campylobacter* spp. are sensitive to oxygen, most media for cultivation are supplemented with whole or lysed blood, FBP (a mixture of ferrous sulphate, sodium metabisulphite and sodium pyruvate), charcoal or hematin plus ferrous sulphate. The bacteria grow better on solid media, if the surface is not dry. The incubation temperature is 37 ºC or 42 ºC, but better is to incubate in the microaerobic atmosphere at 41.5 ± 0.5ºC because these conditions are the optimal for *C. jejuni and C. coli*.

1.2 Overview of *Campylobacter* infections

Campylobacter is considered to be zoonotic bacteria, and with relatively high rate of morbidity and mortality (WHO 2000). *Campylobacter jejuni* strains are the main causes of *Campylobacter* enteritis in humans (Skirrow and Blaser 2000). As low doses as 500 organisms can cause illness to human e.g. accidental ingestion of one drop of raw chicken juice can be easily an infection dose (Newell and Wagenaar 2000). Liu *et al*. (2006) reported that even a very small number of *Campylobacter* cells in food can cause a human infection by *Campylobacter*. Children age of one year and young adults are more susceptible to developing campylobacteriosis (Friedman *et al*. 2004). The notification in humans of campylobacteriosis is mandatory in all member state of EU (EFSA 2015). It is important to mention that the poultry reservoir is responsible for an 80% of human campylobacteriosis cases (EFSA 2010a).

The route from contaminated environment to chicken ceca, further during slaughter from cecal material to poultry carcasses processing, then to other processing stages, and finally to human contain many hurdles, and the exact mechanisms of survival and infection are still poorly understood declareed Bolton (2015). Also, Bolton (2015), mentioned that after ingestion by humans, *C. jejuni* colonises the lower gastrointestinal tract (ileum, jejunum and colon).

Most frequently *Campylobacter* is found in children (Platts-Mills and Kosek 2014). The disease is dependent on the immune status of the host and the virulence of the *Campylobacter* strain, and the symptomatic cases manifest as mild and self- limiting gastroenteritis characterized by fever, vomiting and headaches, followed by abdominal pain with watery or bloody diarrhea reported Bolton (2015). Garénaux *et al.* (2008) mentioned that the campylobacteriosis causes of severe abdominal pain, bloody diarrhea, and sometimes fever.

In the past outbreaks caused by *Campylobacter* have been less prevalent, but nowadays *Campylobacter* gastroenteritis cases are in growing trend, while *Salmonella* outbreaks generally are falling (Gormley *et al*. 2011). In 2016 in the EU 246,307 cases of *Campylobacter* and 94,530 confirmed *Salmonellosis* cases in all member state of EU were reported (EFSA 2016).

Scott *et al.* (2015) found that poultry and some meat products like a liver pate are important sources of *Campylobacter* outbreaks. In other study (Hauri *et al*. 2013), it was suggested that consumption of raw or unpasteurized, or poorly unpasteurized milk have been the reason for increased number of outbreaks of *Campylobacter* infection. Vandeplas *et al.* (2008) suggested that human infections may be caused by direct contact with contaminated animals or animal carcasses contaminated with *Campylobacter*. In the case of production animals as cattle, sheep, goats, pigs and especially poultry, pathogens can spread via the slaughter process to raw and finished products, therefore may cause cross-contamination possibilities at home kitchen level, thereby people may get infection.

1.3 *Campylobacter* spp. transmission routes

1.3.1 Contamination at farm level

Campylobacter spp. are organisms capable of surviving in a wide range of environments, and they have been isolated from rivers, lakes, and waters (Hörman *et al*. 2004; Roasto 2008). The most important primary contamination with *Campylobacter* is taking place at farm level because campylobacters exist widely in the outside environment of rearing halls (Jacobs-Reitsma 2000). According to Cole *et al.* (2004) *Campylobacter* can also colonize the avian reproductive tract and may be vertically transferred between broiler breeder flocks and their offspring. Newell and Fearnley (2003) found that horizontal transmission from environmental sources (wild birds, rodents, water, insects, farm personnel via the boots etc.) is the primary route of *Campylobacter* infection to the poultry farm level.

In the other study by Hiett *et al.* (2002) it was found that epidemiology of *Campylobacter* in broiler production is unknown, but an important source of *Campylobacter* contamination in broiler flocks were surrounding environment of broiler houses. The feed is usually not implicated in the spread of *Campylobacter*, because it is too dry and with too low moisture levels for survival of *Campylobacter* (Newell and Fearnley 2003). Contrary, drinking water can be contaminated by fecal droppings during the rearing period and can serve as important transmission route (Bull *et al.* 2006; Shanker *et al.* 1990).

Study by Bull *et al.* (2006) found that *Campylobacter* can be isolate from air, broiler house, and surroundings of broiler houses. Skov *et al.* (2004), reported that insects could play important role in the *Campylobacter* epidemiology, and as well like mechanical vectors for transmitting the *Campylobacter* to broiler flocks (Ekdahl *et al*. 2005). Vandeplas *et al.* (2008) reported that *Campylobacter* transmission from a contaminated flock to the next flock is not so important, as contaminated litter which can easily be contaminated by *Campylobacter* carrier's broilers fecal droppings and then favor pathogen transmission forward.

Stanley and Jones (2003) mentioned that contaminated litter spread over the agricultural fields can scatter the microorganism in the environment, and then can be attractive for wild birds and insects who after contact with contaminated litter can be infected and then become *Campylobacter* vectors. Bull *et al.* (2006) wrote that *Campylobacter* can survive in the house surroundings soil, and then the farmer can be a vector for *Campylobacter* e.g. via contaminated boots into the broiler house (Newell and Fearnley 2003). Cole *et al.* (2004) reported that *Campylobacter* can also colonize the avian reproductive tract and may be vertically transferred between broiler breeder flocks and their offspring. So, there are multiple ways for the contamination of broiler chicken farms with *Campylobacter* and not all is clear yet enough.
EFSA (2010a) reported that the effective control of *Campylobacter* at the farm level and control of the antimicrobial resistance in poultry meat production chain is the major public health strategy.

1.3.2 Contamination at slaughterhouse level

The primary reservoir of thermophilic *Campylobacter* is gastrointestinal tract of wild and domestic birds and mammals (Roasto 2008). It is known that one of the main reservoirs of *Campylobacter* spp. are broiler chicken, and the colonization level of *Campylobacter* in broiler ceca can reach as high as 10^9 CFU/g (Stern *et al*. 2008). At slaughterhouse level the cross-contamination of broiler chicken meat may occur at scalding, evisceration and water chilling stages, following by the transmission of the *Campylobacter* contamination to the retail level (Stern and Robach 2003).
Carcass contamination usually occurs directly, by leakage of intestinal contents during the initial processing in slaughterhouse (Elvers *et al*. 2011). Franchin *et al.* (2005) found that sources such as cloaca, feathers, coops and breast can be considered factors for cross-contamination with

Campylobacter spp. The contamination of poultry meat, is initially the intestine or gut content which comes to contact with broiler bodies in the broiler house, also direct or indirect contamination may occur during transport and in slaughterhouse environment. Franchin *et al.* (2005) reported that high level of cross-contamination with *Campylobacter* may occur during defeathering and water chilling, and contamination may increase during evisceration, washing and processing of the carcasses. Meremäe *et al.* (2010) found that the prevalence of *Campylobacter* spp. in broiler chickens and the contamination extent at slaughterhouse level is dependent on season, and highest prevalence and numbers of *Campylobacter* can be found in warm summer months.

1.3.3 Prevalence at retail level

Contaminated broiler meat is the major source of human *Campylobacter* infection (Guerin *et al*. 2007). According to Licai *et al.* (2014) *Campylobacter* isolates from retail broiler meats were associated with fecal contamination in the slaughterhouse.
Mäesaar *et al*. (2014) found that broiler chicken meat is the major, but not the only source of *Campylobacter* infection in the human population. Also, it is important to mention that resistant *Campylobacter* strains can be transmitted to humans *via* food chain and could be a risk for human while causing *Campylobacter* infection treatment failures (Hurd *et al*. 2008). Actually, all previously mentioned stages may have a role in the transmission of *Campylobacter* from farm to fork. Chicken meat production and processing chain consist of primary production at rearing farms, transport to slaughter, the slaughter process and subsequent processing of chicken meat products, selling products at the retail level, and handling and consumption of chicken meat products at home and in public places such as restaurants, all of these stages have a role in the transmission of *Campylobacter* from farm to fork (Skarp *et al*. 2015).

1.4. Legislation associated with *Campylobacter* spp.

The aims of EU legislation is to ensure a high level of protection of human health and to decrease incidence of zoonosis in humans, as well protection of animal health and welfare, plant health and environment. The food must not be on the retail level if it is unsafe and unfit for human consumption.

Commission Regulation (EC) No 178/2002 lays down general food law which says that food has to be safe for human consumption, and that responsibility to assure food safety lies on food producers. According to Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, microbiological criteria for foodstuff must be respected by food business operator that accomplishment the general and specific hygiene measures mention in Article 4 of Regulation (EC) No 852/2004.

Samples taken for detection and enumeration of *Campylobacter* spp. according to Commission Regulation (EC) No 2073/2005 of 15 November 2005 must be whole poultry carcasses with neck skin. Samples are taken from broiler carcasses after chilling and the criteria is 1000 cfu/g (m = M; n = 50; c = 20). This regulation lays down the microbiological criteria for certain micro-organisms and the implementing rules to be complied with by food business operators, when implementing the general and specific hygiene measures referred to in Article 4 of Regulation (EC) No 852/2004. To use of the microbiological criteria should be the part of HACCP system based on food production, processing, distribution, and retail level for implementation of good hygiene control measures.

According to the Directive 2003/99/EC on monitoring of zoonoses and zoonotic agents, Member State are obliged to report on *Campylobacter* occurrence or prevalence in food and animals. The notification in humans of campylobacteriosis is mandatory in all member state of EU, but there are exception like France, Belgium, and Italy. In food the notification is compulsory in all members' states.

Nowadays the "farm to fork" is a general principle in EU for food safety and related policy. This is essential to prevent contamination and spread of zoonotic agents. The legislation alone is not able to guarantee the quality and safety of the foodstuff, therefore the main responsibility has to be taken by the farmers, food handlers, and other people working in food production (Roasto 2008).

1.5 Prevention and control of *Campylobacter* contamination

There is no standardized classification exists to describe exactly biosecurity measures (Conan *et al.* 2012). The most efficient primary measures for preventing *Campylobacter* is biosecurity measures and good farm practices to prevent the introduction of *Campylobacter* into a poultry flocks (Rosenquist *et al*. 2003). The most effective steps for preventing *Campylobacter* contamination on the farm level can be divided into three groups (Lin 2009).

1) reduction or elimination of environmental exposure (by biosecurity measures);
2) minimizing the bacterial load (by application of bacteriocins or bacteriophages);
3) improving host resistance (vaccines, probiotics, genetic selection).

According to Meunier *et al*. (2016) reducing the risk of *Campylobacter* infection the biosecurity level should include: disinfection dips for boots, boot change between different poultry houses, and wash hands before and after visits. The author (Meunier *et al.* 2016) it has been reported that *Campylobacter* control strategy include:

1) security and hygiene measures;
2) nutritional strategy;
3) immune strategy.

Hygienic barrier should include:

1) all-in all-out principle;
2) all personnel should wash their hands with soap and water or sanitize them using a disinfectant;
3) change footwear;
4) prevent the entry of insects, rodents, wild birds, and rodent;
5) drinking water should be potable quality;
6) clean and disinfected containers should be always used.

Many studies have been done about nutritional strategies using both *in vitro* and *in vivo* experiments. Those studies suggest that administering in feed or water products like: organic and fatty acids, plant derived products, probiotics, bacteriocins, and bacteriophages which having anti-*Campylobacter* activity, could reduce *Campylobacter* in humans. However, the result of several studies about nutritional products showed there is degradation of the active product before reaching the intestinal tract of chickens, which is the desired site of action (Meunier *et al.* 2016). According to Meunier *et al*. (2016) immune strategy also should be applied at farm level which consist of administering antibodies or vaccine. The author suggested that vaccination is the best strategy, but according to all experiments studies about vaccination the result showed there no enough effective vaccine available to reduce the intestinal *Campylobacter* in chickens.

1.6. Prevalence and occurrence of *Campylobacter* spp. in fresh broiler chicken meat

1.6.1 Baltic countries

In Baltic countries, previously performed studies have shown the difference between Estonia, Latvia, and Lithuania, about *Campylobacter* occurrence in fresh chicken meat. According to Meremäe *et al*. (2010) in Estonia the prevalence of *Campylobacter* in fresh broiler chicken meat from 2000-2002 was 15.8%, and from 2002-2007 the prevalence was 14%, and the *Campylobacter* contamination are more common in summer time, also at the beginning of autumn. Also Estonian Health Board have reported that human campylobacteriosis in Estonia are mostly occurring from June to August while also highest occurrence of *Campylobacter* in poultry meat was found by Meremäe *et al.* (2010). The implementation of strict biosecurity measures at broiler chicken farm level in Estonia, and strictly following the HACCP principle system, and effective hygienic barrier control program, and effective air chilling systems, explains why in Estonia are lower levels of *Campylobacter* contamination compare to other Baltic countries was reported by Meremäe *et al.* (2010). In the other study Kovalenko *et al.* (2013) reported that the prevalence of *Campylobacter* in Latvian broiler chicken samples in 2010 was 59.2% and compare to Estonia this is much higher. Also, according to ESFA (2010a) the *Campylobacter* contamination level of broiler chicken meat in Latvia during the year 2010 was higher from the average of EU member states in 2009. The results of the Kovalenko *et al.* (2013) confirmed that Latvian broiler chicken carcasses and neck skins contain two most prevalent species (*C. jejuni* and *C. coli*) of thermophilic *Campylobacter* which are also related with the most campylobacteriosis cases in humans (EFSA 2010a). Additionally, Kovalenko *et al*. (2013) reported that the *Campylobacter* contamination in Latvia was in early spring, remained high during the summer months and decreased at the end of autumn.

In Lithuania according to Bunevičienė *et al.* (2010) in 2009 *Campylobacter* contamination in fresh broiler chicken meat was more than 40%. Ramonaite *et al.* (2017) also mentioned that the most important source about of human campylobacteriosis in Lithuania is broiler products, and the most important source of *Campylobacter* contamination is taken place at the slaughterhouse level (Kudirkienė *et al.* 2013). Bunevičienė *et al.* (2010) showed that in Lithuania in 2009 the occurrence of *Campylobacter* in chicken wings and drumsticks at the retail level was 46.5%, and for the broiler chicken carcasses at slaughterhouse level it was 45.8%. Meremäe *et al.* (2010) found that the studies done in Lithuania showed seasonal variations of *Campylobacter* to be different than in Estonia. In Lithuania the highest *Campylobacter* occurrence was in winter and spring months, when in Estonia as already mentioned previously it was highest in summer months and in early autumn. Estonian results are similar found in Finland and other Nordic countries (Hänninen *et al*. 2000; Wingstrand *et al*. 2006; Kapperud 1994).

1.6.2 Nordic countries

In the Nordic countries such as Denmark, Finland, Sweden and Norway several studies showed that the higher *Campylobacter* contamination in broiler chicken meat occurred in summer month (Hänninen *et al*. 2000; Wingstrand *et al*. 2006; Kapperud 1994).
The European Food Safety Authority (2005) reported that in many industrialized countries, there has been an increasing incidence of *Campylobacter* during last decades. Hofshagen and Kruse (2005) declared that campylobacteriosis is an important public health problem in most areas of the world, not only in Nordic countries. The risk factors for campylobacteriosis are the consumption of untreated water, eating uncooked meat, or direct contact with infected animals (Kapperud *et al.* 2003).

In Norway science 1990 it was very unfavorable situation regarding campylobacteriosis infection, which required a response from authorities to combat the *Campylobacter* problem. In May 2001 the authorities made plan to fight against *Campylobacter* infections in Norway. The goal of this action plan named as “from stable to table” was to reduce human exposure to *Campylobacter* through Norwegian broiler meat (Hofshagen and Kruse 2005). According to (Hofshagen and Kruse 2005) the results of this action plan during three years from 2002 to 2004, was that the prevalence of positive *Campylobacter* fresh chicken meat declined from 6.3% in 2002; 4.9% in 2003; and to 3.3% in 2004, which represent a 22% reduction from 2002 to 2003, and 33% from 2003 to 2004. Hofshagen and Kruse (2005) mentioned that the seasonal variation was observed, and for all three years the higher prevalence of *Campylobacter* was during the summer time. Reported human cases of campylobacteriosis in Norway were 1,245 to 904 to 2,317 in 2001, 2003 and 2016, respectively. Several studies have reported that the not-disinfected drinking water from natural sources is a major risk factor for acquiring campylobacteriosis in Norway (Kapperud *et al.* 2003; MacDonald *et al.* 2015).

In Denmark human infection with *Campylobacter* spp. from 1992 to 2001 was 21.9 to 86.4 per 100,000 population (Louise 2012). According to Kuhn *et al.* (2017) reported epidemiological data in Denmark *Campylobacter* infection was decreased by 20% in 2000 to 2014, followed by apparently increase from 2014 to 2016. In the other study by MacDonald *et al.* (2015) it was shown that *Campylobacter* infection most frequently in Denmark occur in rural areas, and the source for human infections are mainly farm animals, contaminated water or wildfowl.
The Annual Report on Zoonoses in Denmark (2016), reported that the *Campylobacter* infection increased more than 7% from 2015, and one of the reasons could be that nowadays there are in use more sensitive diagnostic methods compare to past. Present report 2017 by the Danish Technical University’s National Food Institute mention that there were 4,257 cases of *Campylobacter* illness.

In Finland since 1998 campylobacters have been reported to be the cause of intestinal infection in humans (Zoonosis Centre 2012). The *Campylobacter* bacteria are destroyed by heat treatment, and it may spread from uncooked chicken to cooking utensils, which result in cross-contamination to salad or foodstuffs mention (Zoonosis Centre 2012). In the other study

(Hänninen *et al*. 2003) it was mentioned that from 1998 to 2001 seven waterborne outbreaks caused by *Campylobacter jejuni* occurred in Finland. In year 2000 the highest incidence of infection was reported in July until August (Zoonosis Centre 2012), but nowadays according to other Finnish study (Haan *et al.* 2014) it was shown that the most *Campylobacter* infections occur in July and August.

In other study by Mäkeläinen *et al*. (2001) it was mentioned that 90% of population from Finland uses water which are well treated and controlled, but approximately half a million people use water from private wells. According to (Nygård *et al.* 2004) it was found that for the most of *Campylobacter* outbreaks the source was groundwater supplies which were not disinfected. According to National Institute for Health and Welfare (2017) in Finland in 2016 was registered 4,637 causes of *Campylobacter* in humans, and it is more than in 2015. In year 2017 about 4,289 cases (which represent 77.9 cases per 100,000 population) of campylobacteriosis were reported in Finland by EFSA (2018).

In Sweden since year 1991 the *Campylobacter* has been monitored (Hansson *et al*. 2007), but campylobacteriosis was compulsory to declare since year 1989 (SVA 2015). According to (Lindbäck and Svensson 2001) in 1992 in total of 1,453 cases of *Campylobacter* infection was reported, and in year 1999 this number was 2,209. National Veterinary Institute (SVA) in Sweden reported that in 2015 it was increase in number of *Campylobacter* domestic cases (4,709 cases) which form 51% of all 9,180 campylobacteriosis human cases. In the other study Harvala *et al*. (2016) found that the lowest rate of infection is in southwestern parts of Sweden, compare to other regions of the country.

1.6.3 Europe in general

Campylobacter was the most commonly gastrointestinal bacteria pathogen in humans reported by EFSA in 2016 and has been already since from 2005 (EFSA report 2010a; 2015; 2016; 2017). The number of human campylobacteriosis cases in 2013 was 214,779, and in 2016 in EU was 246,307 with 66.3 per 100,000 populations and while comparing years 2016 and 2015 it was

6.1% more *Campylobacter* infection cases in humans than in year 2015. Actually, increased level of campylobacteriosis have been reported every year since 2005 in EU.

According to EFSA from 2012 to 2016, twelve member state including Lithuania, Latvia, and Sweden reported increased level of *Campylobacter* infection, but in Estonia no significant changes was seen compared to previous year (table 1). The highest rate of *Campylobacter* infection in 2016 was in Czech Republic with 228.2 cases per 100,000 peoples at total of 24,291 human cases, followed by Slovakia and Sweden with 111.9 cases per 100,000 people, a total of 11,021 human cases were reported. Recent EFSA report (2018), informed that the number of human campylobacteriosis cases in 2017 in EU was lower compared with 2016 year, the only country where it was higher was Czech Republic with 230.0 cases per 100,000 people.

The broiler meat is considered to be an important source for human diseases. The infection resulted mostly from undercooking of poultry meat or cross-contamination of other foods by raw poultry meat. Thorough cooking of broiler meat and strict kitchen hygiene, and proper refrigeration temperatures would prevent or reduce the risk by *Campylobacter* contamination.

Table 1. Reported total cases and notification rates of human campylobacteriosis per 100,000* in the EU/EEA, 2013-2017.

Year	Countries						
	Estonia	Latvia	Lithuania	Norway	Sweden	Finland	Denmark
2013	382	9	1,139	3,291	8,114	4,066	3,722
2014	285	37	1,184	3,386	8,288	4,889	3,733
2015	318	74	1,186	2,318	9,180	4,588	4,327
2016	382	93	1,225	2,317	11,021	4,637	4,712
2017	347	61	993	3,884	10,608	4,289	4,255
2013	28.9	0.4	38.3	65.2	84.9	74.9	67.3
2014	21.7	1.8	40.2	66.3	85.9	89.7	67.0
2015	24.2	3.7	40.6	44.9	94.2	83.8	76.5
2016	22.6	4.6	42.4	44.5	111.9	84.5	82.6
2017	21.7	3.0	34.8	73.9	106.1	77.9	74.0

*the lower part of the table indicates notification rates per 100,000 inhabitants

2. AIMS OF THE STUDY

The aim of the study was to investigate *Campylobacter* spp. prevalence in fresh broiler chicken meat of Estonian, Latvian and Lithuanian origin purchased at Estonian retail level.

Second aim of the present work was to study the numbers of *Campylobacter* spp. in the same fresh broiler chicken meat samples.

3. MATERIALS AND METHODS

3.1 Sample collection

The samples were collected from the biggest Estonian supermarket retail outlets in Tartu where meat is sold for domestic consumption. Estonia, Latvia, Lithuania represent the most common origins of the broiler chicken meat available in the Estonian retail market. A total of 249 fresh broiler chicken meat samples were collected on a monthly basis between September 2018 and March 2019. From each country of origin 83 samples were collected and 166 analyses were performed. Both detection and enumeration methods were used, therefore altogether 498 analyses were performed (table 2). Packaged products such as broiler chicken thighs, legs, half-legs, breast and wings were collected at retail level included the biggest food retail outlets such as “Coop Maksimarket”, “Rimi”, “Maxima” and “Prisma” in Tartu town. Samples were transported to the laboratory in a portable cooler and kept at appropriate refrigerated temperatures before analyses were made. All the analyses were performed in microbiology laboratory of the Chair of Food Hygiene and Veterinary Public Health of the Estonian University of Life Sciences.

Table 2. Number of samples and analyses

Origin	No. of samples	Analyses		Total No. of analyses
		Enrichment method	Enumeration method	
Estonia	83	83	83	166
Latvia	83	83	83	166
Lithuania	83	83	83	166
In total	249	249	249	**498**

3.2 *Campylobacter* spp. enumeration

Campylobacter spp. enumeration and detection from the meat samples was performed according to the International Organization for Standardization method described in ISO 10272–1:2017. According to mentioned ISO method for enumeration, 10 g of chicken skin was aseptically taken from chicken legs and placed into a sterile plastic bag. Thereafter 90 ml of buffered Peptone water was poured into the bag, and the sample were homogenized during one minute in a stomacher. By following 0.1 ml of ten-fold dilution material was taken and carried onto the surface of two mCCD (Oxoid; Basingstoke, Hampshire, UK) agar plates. Plates were incubated in a microaerobic atmosphere (Oxoid Ltd, Basingstoke: CampyGen™2.5L) at 41.5 °C ±0.5°C, for 48 h (Figure 1).

3.3 *Campylobacter* spp. detection

Detection of *Campylobacter* spp. was done by using enrichment method, according to ISO standard. For it 10 g of chicken skin was aseptically taken from the chicken legs, and placed into a sterile Duran bottle (volume 100 ml), then 90 ml of Preston enrichment broth were added into bottle, and bottles were incubated in a microaerobic atmosphere at 41.5 ± 0.5 °C, for 24 ± 2 h. After 24 hour of incubation 10 µ loopful of Preston enrichment material was inoculated onto mCCD agar (Oxoid; Basingstoke, Hampshire, UK), plate. Plates were incubated in a microaerobic atmosphere by using anaerostate together with CampyGen™ 2.5L reagent envelopes (Oxoid Ltd, Basingstoke, UK) at 41 ± 0.5 °C, for 44 ± 2 h, see Figure 1.

3.4 Identification colonies of *Campylobacter* spp.

After incubation on mCCD agar the typical *Campylobacter* colonies were seeked. Typical *Campylobacter* colonies are silver-greyish on mCCD agar, with a metallic sheen, and bacteria are flat and moist, with a tendency to spread. Typical colonies were counted according to enumeration method and further colonies were streaked onto Columbia blood agar (Oxoid Ltd: Hampshire, UK) plates, which were incubated for 48 h at 41.5 ± 0.5 °C in microaerobic conditions. After incubation the confirmation was performed with bacterial growth material obtained from Columbia blood agar. *Campylobacter* colonies were examined for characteristic morphology and motility using a microscope, additionally gram staining was performed. On the microscope the typical *Campylobacter* bacteria were like small curved bacilli with typical spiraling “corkscrew” motility. By examination with gram staining *Campylobacter* spp. are negative bacteria and are colored pink with typical morphology to *Campylobacter* spp. After the isolation, the *Campylobacter* strains were stored at –80°C in glycerol broth (20% [vol/vol] glycerol in 1% [wt/vol] proteose peptone) for further studies.

Following figure (Figure 1) is shortly illustrating enumeration (A) and detection (B) method.

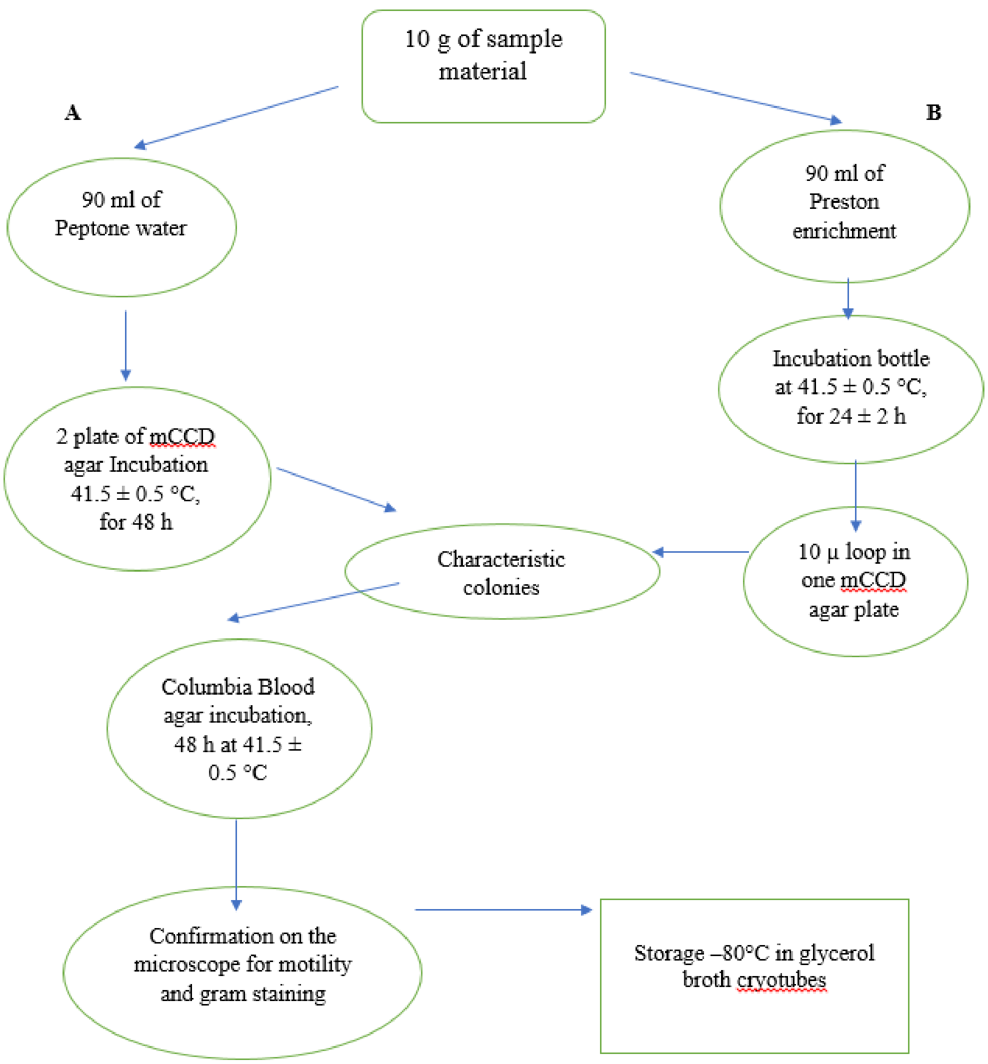

Figure 1: Method for detection and enumeration of *Campylobacter* spp.

3.5. Statistical analysis

The prevalence and confidence intervals (CI) based on probabilities derived from binominal distribution for proportion were calculated using the software VassarStats (Lowry 2019). The statistical significance of the difference in prevalence between different groups was investigated using both Chi-square test and Fisher exact test. A p-value < 0.05 for both tests was considered statistically significant.

4. RESULTS

***Campylobacter* prevalence**

Table 3 shows the prevalence of *Campylobacter* spp. in broiler chicken meat samples between September 2018 and March 2019. *Campylobacter* spp. was isolated in 77 (30.9%) of 249 broiler chicken meat samples. Altogether, 28 (33.7%) of Latvian origin and 49 (59%) of Lithuanian origin fresh broiler chicken meat samples were positive for *Campylobacter* spp. at Estonian retail level. All 83 broiler chicken meat samples of Estonia origin were negative for *Campylobacter* spp.

All those samples that were *Campylobacter*-positive by an enumeration method were also *Campylobacter*-positive using a selective enrichment method. However, a total of 77 (30.9%) positive samples were detected by a selective enrichment method, while 39 (15.7%) positive samples were detected by enumeration method. It can be explained by the fact that enrichment of broiler chicken meat samples allows the detection of any viable culturable *Campylobacter* spp. cell in sample, while the quantification limit (the threshold) for the enumeration method is 100 CFU/g.

Table 3. Prevalence of *Campylobacter* spp. in fresh broiler chicken meat samples of Estonian, Latvian and Lithuanian origin at Estonian retail level

Origin	No. of samples	No. of positive samples / positive %	CI95% of positive %
Estonia	83	0 / 0	0 – 5.5
Latvia	83	28 / 33.7	24.0 – 45.0
Lithuania	83	49 / 59.0	47.7 – 69.5
Total	249	77 / 30.9	25.3 – 37.1

A total of 172 (69.1%) broiler chicken meat samples were *Campylobacter* spp. negative in our study.

The following table is illustrating the statistical differences in *Campylobacter* prevalence in fresh broiler chicken meat in comparison of country of origin.

Table 4. Statistical analysis of *Campylobacter* prevalence's dependent on country of origin of analyzed fresh broiler chicken meat products

Compared groups	Statistical analyses method	
	Chi-Square Test, P value	Fisher Exact Probability Test two-tailed, P value
Latvian *versus* Lithuanian	0.002	0.002
Estonian *versus* Latvian	<0.0001	<0.0001
Estonian *versus* Lithuanian	<0.0001	<0.0001

Chi-square test of association is used if the sample size is not too small, and Fisher exact probability test is used if the sample size is not too large. In our case both methods were used because sample size is not too small, either it is not large.

Differences in *Campylobacter* prevalence's between samples of country of origin in two groups (e.g. Estonian *versus* Latvian) were statistically analysed are shown in table 4.

Campylobacter numbers

The distribution of *Campylobacter* numbers for the 77 positive broiler chicken meat samples is shown in table 5. Among *Campylobacter*-positive samples, 38 (15.3%) of the samples contained campylobacters below 100 CFU/g. Only one (1.2%) sample of Latvian origin and a total of 17 (20.5%) samples of Lithuanian origin contained *Campylobacters* between 100 and 499 CFU/g. The other nine (10.8%) samples of Lithuanian origin contained *Campylobacter* counts of between 500-1,000 CFU/g.

Table 5. *Campylobacter* numbers in fresh broiler chicken meat

Origin	*Campylobacter* numbers (CFU/g)				
	0*	<100**	100-499	500-1,000	>1,000
Estonia	83 (100.0)	0 (0)	0 (0)	0 (0)	0 (0)
Latvia	55 (66.3)	25 (30.1)	1 (1.2)	1 (1.2)	1 (1.2)
Lithuania	34 (41.0)	13 (15.7)	17 (20.5)	9 (10.8)	10 (12.0)
Total	**172 (69.1)**	**38 (15.3)**	**18 (7.2)**	**10 (4.0)**	**11 (4.4)**

number of samples (percentage)

*negative detection and negative enumeration

**negative enumeration and positive detection, the threshold

Campylobacter contamination seasonality

Figure 2 gives an overview about the seasonality of *Campylobacter* spp. contamination on fresh broiler chicken meat of Estonian, Latvian and Lithuanian origin in defined study period from September 2018 to March 2019. No distinct seasonal variation in *Campylobacter* spp. contamination was observed. High occurrence of *Campylobacter* spp. contamination in broiler chicken meat samples of Lithuania origin was in October 2018 and from December 2018 to March 2019. During these months, 60.0-85.7% of samples were positive for *Campylobacter* spp. For comparison, the seasonal peak of *Campylobacter* contamination in tested samples of Latvian origin was in September and November 2019, when 85.7% and 61.9% of the samples were positive, respectively. All broiler chicken meat samples of Estonia origin irrespective of the month were negative for *Campylobacter* spp.

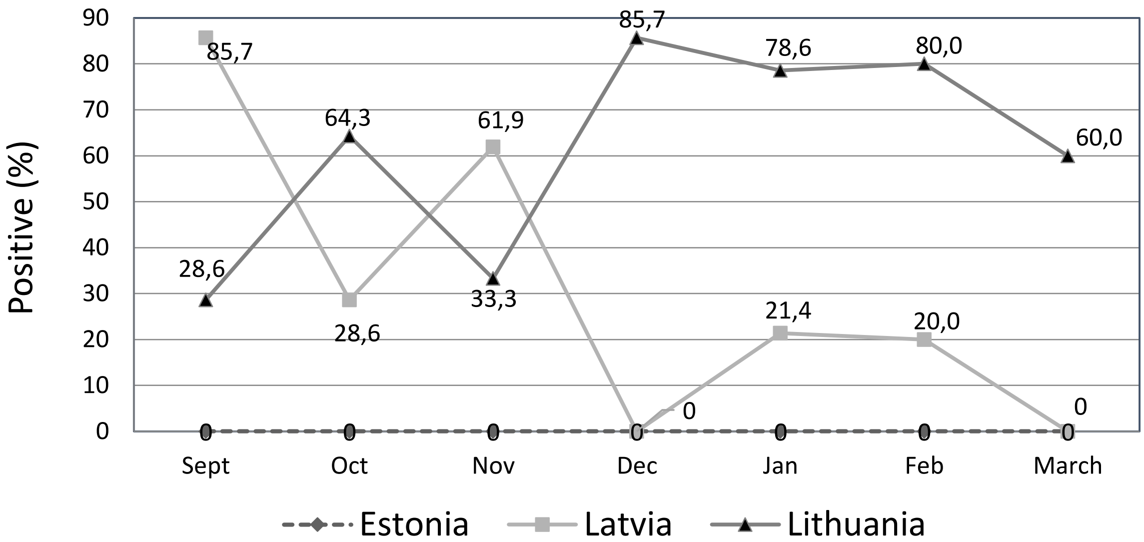

Figure 2. Proportion of *Campylobacter* positive fresh broiler chicken meat samples within different months in 2018 and 2019

Campylobacter contamination within trade mark

Figure 3 provides an overview of the links between *Campylobacter* spp. contamination on fresh broiler chicken meat and the product trade mark. *Campylobacter* spp. were isolated in 43 of 71 (60.6%) of broiler chicken meat samples of Lithuanian origin sold under "Rannamõisa" trade mark at Estonian retail level. Of 63 broiler chicken samples of Latvian origin sold as "Kekava" trade mark, *Campylobacter* spp. were found in 26 (41.3%) samples. Trade mark "Talupoja" included broiler chickens raised in Lithuanian farms, transported to the Latvia and slaughtered in the Latvian slaughterhouse No A002845, and sold in Estonia. Although the number of tested samples was relatively small, *Campylobacter* spp. was isolated in 8 (80%) of the "Talupoja" products. All tested broiler chicken meat products with trade mark "Tallegg" and "Goodlife" were negative for *Campylobacter* spp.

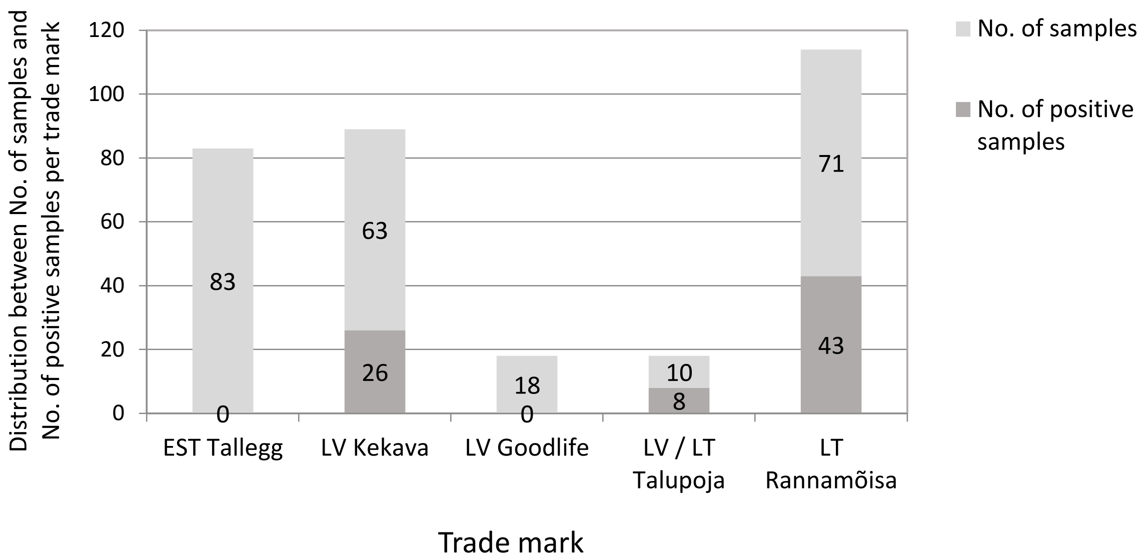

Figure 3. *Campylobacter* spp. contamination proportions of fresh broiler chicken meat of Estonian, Latvian and Lithuanian origin according to trade mark

Campylobacter contamination within slaughterhouse

Figure 4 gives an overview about *Campylobacter* spp. contamination on fresh broiler chicken meat dependence on slaughterhouse. The findings showed that these broiler chickens, which originated from the Estonian farms named as Rannamõisa, Kaarma, Laabi, Saha, Kumna and Loo, were negative for *Campylobacter* spp. at farm as well as at slaughterhouse level, because broiler chicken meat samples collected in present study were all negative for *Campylobacter* spp.. *Campylobacter*-positive broiler chicken meat samples were probably associated with *Campylobacter*-positive broiler chickens in the farms and the spread of contamination at slaughterhouses No. A002845, 41-28 and 49-01. *Campylobacter* spp. were isolated in 5 of 5 (100%) broiler chicken meat samples originated from slaughterhouse No. 49-01 followed by slaughterhouse No. 41-28 and No. A002845. From 75 and 86 of broiler chicken meat samples originated from the last mentioned slaughterhouses, 57.3% and 33.7% were positive for *Campylobacter* spp., respectively.

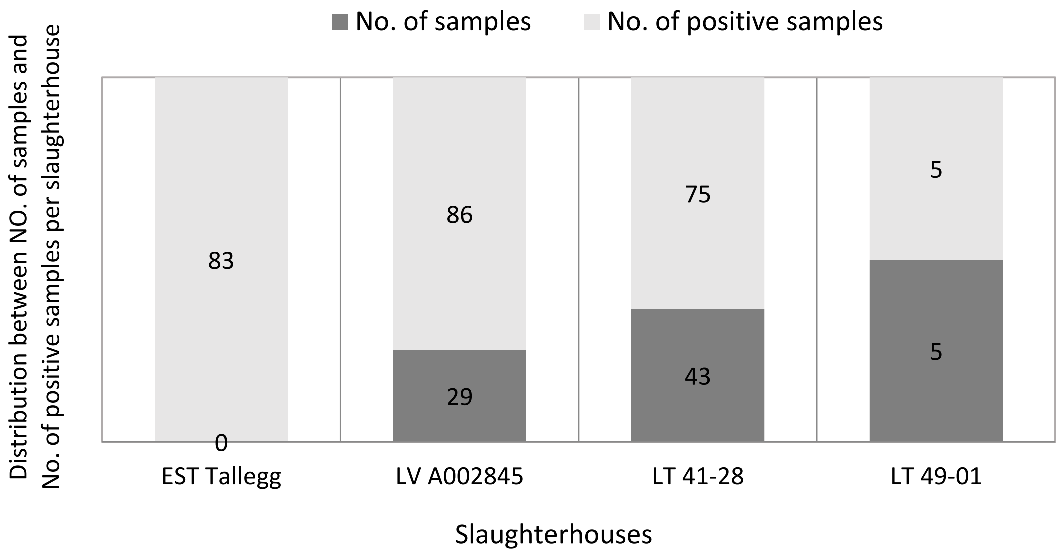

Figure 4. *Campylobacter* spp. contamination on fresh broiler chicken meat of Estonian (EST), Latvian (LV) and Lithuanian (LT) origin depends on slaughterhouse (i.e. information on package)

Campylobacter contamination within sample site

Figure 5 gives an overview about *Campylobacter* spp. contamination on fresh broiler chicken meat of Estonian, Latvian and Lithuanian origin dependent on sample site. This study was not designed to evaluate connections between *Campylobacter* spp. contamination and sample site. However, among *Campylobacter*-positive samples, the broiler chicken half-legs and legs, followed by thighs were contaminated at high level, when a total of 36.8% (39/106), 33.3% (28/84) and 19.2% (10/52) of the analyzed fresh broiler chicken meat samples were positive at Estonian retail level, respectively.

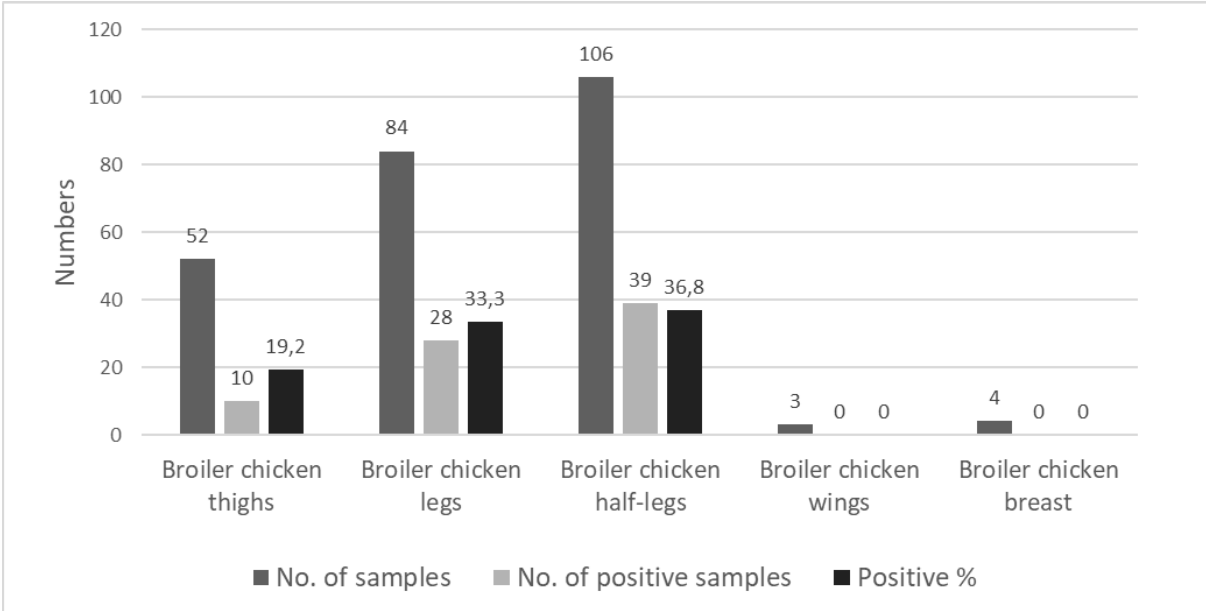

Figure 5. *Campylobacter* spp. contamination on fresh broiler chicken meat dependent on sampling site

5. DISCUSSION

***Campylobacter* prevalence**

In present study it was found that *Campylobacter* prevalence was significantly ($p < 0.0001$) lower in Estonian fresh broiler chicken meat products compared to Latvian and Lithuanian origin products. Also, *Campylobacter* prevalence in Latvian fresh broiler chicken meat products was lower than in Lithuanian origin fresh broiler chicken meat products. Similar findings have been found from previous studies performed in Estonia. There are similarities with earlier study by Mäesaar *et al.* (2014) who found that the prevalence of *Campylobacter* spp. in Estonian origin fresh broiler chicken meat products was significantly lower ($p < 0.001$) than in Latvian and Lithuanian same type of broiler chicken meat products. Mäesaar *et al.* (2014) reported that *Campylobacter* contamination at retail level in Estonia on fresh broiler chicken meat in Latvian and Lithuanian fresh broiler chicken products was 60% and 50%, respectively. Compared with earlier Estonian studies it can be deduced that the *Campylobacter* prevalence in Estonian origin products has continuously decreased, and in present study not any sample was *Campylobacter* positive. The reason for this is unknown, but we can speculate that more strict biosecurity and biosafety measures are applied in Estonian broiler chicken production in recent years.

Previous study by Bunevičienė *et al*. (2010) showed that in Lithuania in 2009 the occurrence of *Campylobacter* in chicken wings and drumsticks at the retail level was 46.5%. In present study we found that 59% of Lithuanian fresh broiler chicken products were Campylobacter positive. This means that *Campylobacter* spp. prevalence in Lithuanian fresh broiler chicken products has previously been high and still is very high.

In Latvian products the proportion of *Campylobacter* spp. positive products in present study was 33.7%. This is lower contamination compare to 60.0% found in Mäesaar *et al*. (2014) study for Latvian products at Estonian retail, but in Mäesaar *et al.* (2014) study all 12 months were included to the *Campylobacter* spp. prevalence study. In present study only the period from September to March was studied. Similarly to Mäesaar *et al*. (2014) also Kovalenko *et al*. (2013) reported that the occurrence of *Campylobacter* in Latvian retail level was 59.2%. It does mean that Latvian products are more contaminated with *Campylobacter* than Estonian products.

Campylobacter numbers

The results showed that more than 1000 CFU/g contained a total of one (1.2%) of Latvian and 10 (12.0%) of Lithuanian origin fresh broiler chicken meat products. Among these *Campylobacter*-positive sample of Latvian origin was collected in February 2019 and contained 1500 CFU/g. The highest count of *Campylobacter* spp. (4500 CFU/g) was detected in one broiler chicken meat sample of Lithuanian origin in January 2019. Enumeration results are indicating that Lithuanian products may pose higher risk for human campylobacteriosis than Estonian and Latvian ones. High prevalence and high numbers of *Campylobacter* spp. in fresh broiler chicken meat are the risk factors for human campylobacteriosis (Mäesaar *et al.* 2014). Together with previous Lithuanian (Bunevičienė *et al*. 2010) and Estonian study (Mäesaar *et al*. 2014) results where *Campylobacter* prevalence and numbers were determined, it can be suggested that *Campylobacter* control programs for broiler chicken meat production should be initiated in Lithuania. More efforts should be made to lower *Campylobacter* numbers as well as prevalence in broiler chicken meat, especially because quite a lot of the fresh broiler chicken meat is exported from Lithuania to Latvia and Estonia.

Seasonality in Campylobacter contamination

High occurrence of *Campylobacter* spp. contamination in broiler chicken meat samples of Lithuania origin was in October 2018 and from December 2018 to March 2019. Meremäe *et al.* (2010) has described previous Lithuanian studies were the highest *Campylobacter* occurrence was found in winter and spring months. This is very interesting finding because December, January and February are the coldest months of the year. Usually in most of the other countries the *Campylobacter* contamination is lowest during winter time. This kind of seasonal phenomenon need further studies to get more information why in Lithuanian broiler chicken products the contamination is so high during winter. As mentioned previously not all months of the year were studied in present work. Therefore, the seasonal impact to the *Campylobacter* contamination in fresh chicken meat cannot be properly estimated. In Latvia, Kovalenko *et al*. (2013) reported that *Campylobacter* contamination in Latvia was highest in early spring, remained high during the summer months and decreased at the end of autumn. In our study we find that the seasonal peak of *Campylobacter* contamination in analyzed samples of Latvian origin was in September and November 2019, when 85.7% and 61.9% of the samples were positive. In earlier study of Meremäe *et al*. (2010) it was found that *Campylobacter* contamination in Estonia was more common in summer time, also at the beginning of autumn.

Sample site

Sample site mean different anatomical areas where from the fresh broiler chicken meat (meat cuts) samples are originating e.g. wings, thighs, breast and legs. Because mostly legs, half-legs and thighs (upper part of leg) were studied for *Campylobacter* contamination, the results of present work are not adequate to estimate the real contamination differences dependent on sample site. Latter is because this study was not designed to estimate differences of *Campylobacter* contamination of different anatomical regions of poultry carcasses. However, it was found that most contaminated samples were broiler chicken half-legs (36.8%), followed by broiler chicken legs (33.3%) and chicken thighs (19.2%).

Finally, EFSA (2018) latest zoonosis document reported that in Lithuania, Latvia and Estonia respectively 993, 61 and 347 of human campylobacteriosis cases were registered in year 2017. Unfortunately, in Estonia the *Campylobacter* enteritis cases are in rising trend. This means that more efforts should be given to contamination prevention from farm to fork. Also consumers should know better how to prevent cross-contamination at home kitchens. Also, according to present study results Estonian consumers should prefer Estonian fresh broiler chicken meat products to imported ones, because in present study all Estonian products were found to be negative for *Campylobacter* spp. by using both detection and enumeration method. It is more probable to get *Campylobacter* infection from other sources than from Estonian fresh broiler chicken meat in Estonia. This was also concluded by Mäesaar *et al*. (2014).

6. CONCLUSION

High prevalence of *Campylobacter* on fresh broiler chicken meat of Latvian and Lithuanian origin was found in the Estonian retail market. *Campylobacter* was not found from fresh broiler chicken meat of Estonian origin. Generally, there was no distinct seasonal peak found for *Campylobacter* contamination of fresh chicken meat during study period, but interestingly for Lithuanian fresh broiler chicken meat products the contamination was highest in December, January and February.

The greater *Campylobacter* prevalence and numbers of broiler chicken meat of Latvian and Lithuanian origin might pose greater *Campylobacter* exposure risks to the Estonian population compare to the campylobacteriosis risk from Estonian origin fresh broiler chicken meat products for which the *Campylobacter* contamination was not detected during 7 months study period.

SUMMARY

Fresh broiler chicken meat is the most important source of *Campylobacter* spp. which causes campylobacteriosis, mostly with enteric infection symptoms in human. In the Nordic countries such as Finland, Sweden and Norway one of the most important source for human camplobacteriosis beside of poultry meat is also not properly treated ground water.

In Estonia, compared with most EU-country and other Baltic countries the prevalence of *Campylobacter* is relatively low. It is probably because in Estonia broiler chicken farms apply strict biosafety and biosecurity measures at farm level as well as self-control including HACCP principle at slaughterhouse and meat industry level. It has been found that in Estonia humans get contaminated with *Campylobacter* mostly because of eating broiler chicken meat and within traveling abroad where general hygiene incl. food hygiene levels are often much lower than in Estonia.

In present work *Campylobacter* spp. contamination was studied in Estonian, Latvian and Lithuanian origin fresh broiler chicken meat at Estonian retail level. All the meat samples were company packaged which eliminated the possibility for contamination at retail level. Main findings of present work suggest that there is higher campylobacteriosis risk by consuming Lithuanian origin fresh broiler chicken meat products than those originating from Estonia and Latvia. Latter is derived from fact that both *Campylobacter* prevalence and numbers were significantly higher in Lithuanian origin fresh broiler chicken meat compare to Estonian and Latvian origin samples during study period from September 2018 to March 2019. Because not all months of the year were studied in present work, it cannot be properly estimated the seasonal impact to the *Campylobacter* contamination in fresh chicken meat, but it was very interesting finding that in Lithuanian products the *Campylobacter* contamination was highest in December, January and February. These months represents the coldest months of year while usually in most of the other countries the *Campylobacter* contamination is lowest. This phenomenon need to be further investigated, probably together with Lithuanian colleagues in the near future.

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Annex 1

Some examples of sample preparation and analyses performed by author

Annex 2

Typical *Campylobacter* colonies (here after enrichment) are silver-greysh on mCCD agar.